Amendments to the Specification

Please amend the paragraph beginning at page 39, 15 as follows:

B. Construction of Transgenic Potato with TGEV S Gene A DNA fragment containing the A, B, C and D antigenic sites of the S gene of TGEV was isolated by PCR amplification and gel purification. The oligonucleotides had the following sequences GG**CCATGG**CTAAACTATTTGTGGTTTTGGTCG (SEQ ID NO:1) and TCTC**GAGCTC**TTATTATTAAGCACCAACAGGACTCAACGAC (SEQ ID NO:2), the sequences are in the 5' to 3' direction (with restriction and nuclease restriction sites shown in bold. The 1929 nucleotide PCR fragment was cleaved using standard techniques with the restriction enzymes Nco1 and Sac1. The Ncol/Sacl cleaved PCR fragment was ligated into a modified shuttle vector derived from p101[72] using standard techniques well known in the art. The DNA construct was named NS-p101. NS-p101 was transformed into *Agrobacteria* and used to transfect potato plants. The resulting potato strain was named 67-TGN101.7.

Page 25, line 2, please amend the paragraph as follows:

Leaf disc transformation was performed in accordance with the procedure of Horsch et al[6]. Tomato and tobacco seedlings were grown in flats under moderate light and temperature and low humidity to produce uniform, healthy plants of ten to forty centimeters in height. New flats were started weekly and older plants were discarded. The healthy, unblemished leaves from the young plants were harvested and sterilized in bleach solution containing ten per cent (10%) household bleach (diluted one to ten from the bottle) and one tenth per cent (0.1%) ~~Tween~~ TWEEN 20 or other surfactant for fifteen to twenty minutes with gentle agitation. The leaves were then rinsed

three times with sterile water. The leaf discs were then punched with a sterile paper punch or cork borer, or cut into small strips or squares to produce a wounded edge.

Page 25, line 33, please amend the paragraph as follows:

Root, stem, leaf and fruit samples of the plants were excised. Each tissue was homogenized in a buffered solution, e.g. one hundred millimolar sodium phosphate (100 mM), pH 7.4 containing one millimolar ethylenediamine tetraacetate (1.0 mM EDTA) and five-tenths millimolar phenylmethylsulfonyl fluoride (0.5 mM PMSF) as a proteinase inhibitor. The homogenate was centrifuged at five thousand times gravity (5000XG) for ten minutes. A small aliquot of each supernatant was then reserved for protein determination by the Lowry method. The remaining supernatant was used for the determination of the level of HBsAg expression using two standard assays: (a) a HBsAg radioimmunoassay, the reagents for which were purchased from Abbott Laboratories and (b) immunoblotting using a previously described method of Peng and Lam[61] with a monoclonal antibody against anti-HBsAg purchased from ~~Zymed~~ ZYMED Laboratories. Depending upon the level of HBsAg expression in each tissue, the supernatant may have been partially purified using a previously described affinity chromatographic method of Pershing et al[62] using monoclonal antibody against HBsAg bound to commercially available ~~Affi-Gel~~ AFFI-GEL10 gel from ~~Bio-Rad~~ BIO-RAD Laboratories, Richmond, CA. The purified supernatant was then concentrated by lyophilization or ultrafiltration prior to radioimmunoassay and immunoblotting.

Page 27, line 26, please amend the paragraph as follows:

The HBsAg DNA coding sequence[64,65] (the S gene) was excised from plasmid pMT-SA (constructed at Chinese Academy of Sciences) as a Pst I-Hind III fragment and isolated by electrophoresis in a one percent (1%) agarose gel. The Pst-Hind III fragment was visualized in the agarose gel by staining with ethidium bromide, illuminated with ultraviolet light (UV) and purified with a Prep-a-Gene kit (~~BioRad~~ BIO-RAD Laboratories, Richmond, CA). The HBsAg coding region on the Pst I-Hind III fragment was then ligated into the Pst I-Hind III digested plasmid pBluescript KS (Stratagene, La Jolla, CA) to form the plasmid pKS-HBS. The HBsAg gene in plasmid pKS-HBS was then opened 116 base pairs (bp) 3' to the termination codon with BstB I and the resulting ends were blunted by filling with Klenow enzyme and dCTP/dGTP. The entire coding region (820 bp) was then excised with Bam HI, which is site derived from the plasmid vector pBluescript. This results in the addition of Bam HI and Sma I sites 5' to the original HBsAg coding sequence from plasmid pMT-SA.

Page 32, line 19, please amend the paragraph as follows:

Protein was extracted from transformed tobacco leaf tissues by homogenization with a Ten-Broek ground glass homogenizer (clearance 0.15 mm) in five volumes of buffer containing twenty millimolar (20mM) sodium phosphate, pH 7.0, one hundred fifty millimolar (150mM) sodium chloride, twenty millimolar (20mM) sodium ascorbate, one-tenth percent (0.1%) Triton X-100, and five tenths millimolar (0.5mM) PMSF, at four degrees Celsius (4°C). The homogenate was centrifuged at one thousand times gravity (1000XG) for five minutes and the supernatant centrifuged at twenty-seven thousand times gravity (27,000XG) for fifteen minutes. The 27,000XG

supernatant was then centrifuged at one hundred thousand times gravity (100,000XG) for one hour and the pellet resuspended in extraction buffer. The protein in the different fractions was measured by the Coomassie dye-binding assay (~~Bio-Rad~~BIO-RAD Laboratories). HBsAg protein was assayed by the AUSZYME Monoclonal kit (Abbott Laboratories, Abbott Park, IL) using the positive control, HBsAg derived from human serum, as the standard. The positive control was diluted to give HBsAg protein levels of nine hundredths to one and eight tenths nanograms (.09-1.8 ng) per assay. After color development, the absorbance at four hundred ninety-two nanometers (492 nm) was read and a linear relationship was found. As seen in Figure 6B, the weld-type control plant contained no detectable HBsAg protein (Column 1); fairly low levels of HBsAg protein were observed, ranging from three to ten nanograms per milligram (3-10ng/mg) soluble protein for the pHB101 construct (Columns 2 through 6); and from twenty-five to sixty-five nanograms per milligram (25-65 ng/mg) for the pHB102 construct (Columns 7 through 9). The reaction was specific because the wild-type tobacco showed no detectable HBsAg protein. HBsAg from human serum and recombinant HBsAg (rHBsAg) from plasmid-transformed yeast occur as approximately twenty nanometer (20nm) spherical particles consisting of protein embedded in a phospholipid bilayer. Ninety-five percent of the rHBsAg in the 27,000XG supernatants of transgenic tobacco leaf extracts pelleted at 2000,000XG for thirty minutes. This suggested a particle form. Thus, evidence was sought to ascertain if rHBsAg in tobacco existed as particles.

Page 33, line 16, please amend the paragraph as follows:

Monoclonal antibody against HBsAg, clone ZMHB1, was obtained from ~~Zymed~~ ZYMED Laboratories (South San Francisco, CA). The immunogen source for this antibody is human serum.

The monoclonal antibody was bound to ~~Affi-Gel~~ AFFI-GEL HZ hydrazide gel (~~Bio-Rad~~ BIO-RAD Laboratories, Richmond, CA) according to the instruction supplied in the kit. The 100,000XG resuspended soluble material was made to five tenths molar (0.5M) sodium chloride and mixed with the immobilized antibody-gel by end-over-end mixing for sixteen hours at four degrees Celsius (4°C). The gel was washed with ten volumes of PBS.5 [ten millimolar (10mM) sodium phosphate, pH 7.0, five tenths molar (0.5M) sodium chloride] and ten volumes of PBS.15 [fifteen hundredths molar (0.15M) sodium chloride] and bound HBsAg eluted with two tenths molar (0.2M) glycine, pH 2.5. The eluate was immediately neutralized with Tris-base, and particles pelleted at one hundred and nine thousand times gravity (109,000XG) for one and a half hours at five degrees Celsius (5°C). The pelleted material was negatively stained with phosphotungstic acid and visualized with transmission electron microscopy using a Phillips CMIO microscope. The presence of rHBsAg particles were revealed by negative staining and electron microscopy, Figure 7. rHBsAg particles ranged in diameter between ten and forty nanometers (10-40nm). Most particles were between sixteen and twenty-eight nanometers (16-28nm). These are very similar to the particles observed in human serum,[69] although no rods were observed. The rHBsAg particles from yeast occur in a range of sizes with a mean of seventeen nanometers (17nm).[2] Thus rHBsAg produced in transgenic tobacco leaves has a similar physical form to the human HBsAg.

Page 38, line 2, please amend the paragraph as follows:

Leaves of transformed or untransformed tomato plants were excised and pressed on fine-grain sandpaper before blotting abaxial side down on nitrocellulose. Tomato fruits were sectioned with a razor blade and pressed onto nitrocellulose for 30 sec. The blot was blocked with 5% nonfat dry milk in 10 mM sodium phosphate, pH 7.2, 140 mM NaCl, 0.05% ~~Tween~~TWEEN-20, 0.05% NaN3 (PBST)for 2 hr at 37°C. The blot was probed with mouse monoclonal anti-HBsAg (~~Zymed~~ ZYMED Laboratories) at 1:1000 dilution in 2% nonfat dry milk in PBST for 2 hr at 23°C, before washing and detection with goat anti-mouse IgG-alkaline phosphatase conjugate (~~BioRad~~BIO-RAD Laboratories) and development with NBT and BCIP according to manufacturer's instructions (Genius 2 Kit, Boehringer-Mannheim).

Page 41, line 7, please amend the paragraph as follows:

I. Detection of TGE virus from clinical samples by indirect immunofluorescent Assay (IFA). Dilutionmedium or maintenance medium consisted of Eagles minimal essential medium (MEM) with nonessential amino acids, 200 mM L-glutamine, 50 ug/ml Gentamycin, 10 ug/ml trypsin, 0.01% DEAE-Dextran, 0.02 M Hepes at pH to 7.2. Test samples were supernatants from centrifugation at 2000 x g for 20 minutes, where the ~~sharting~~ starting materials were either fecal samples, ~~intentinal~~intestinal contents, or extracts. ST cells were grown to confluence in 4-well ~~Lab-Tek~~ LAB-TEK chamber slides (Nunc. # 177399). TGE SRV (Ambico Inc. SRV-7) Swine anti-TGEV hyperimmune serum was also used. Optimal dilution used for IFA staining was predetermined for each lot. Fluorescein-labeled affinity purified antibody to swing IgG (H+L) was obtained. Optimal dilution used for IFA staining was also predetermined for each lot.

Page 41, line 17, please amend the paragraph as follows:

The confluent ST cell monolayer in each ~~Lab-Tek~~ LAB-TEK slide chamber was rinsed 3 times with dilution medium (1 ml/rinse). The last rinse was left on for at least 30 minutes. Test samples were diluted to 1:10 and 1:100 in the dilution medium, and inoculated into 2 chambers/dilution with 0.2 ml/chamber. For control slides, 1 chamber of cells was inoculated with dilution and maintenance medium (Negative control), and 3 chambers were inoculated with 0.2 ml of 10^{-4}, 10^{-5}, and 10^{-6} diluted TGE SRV with 1 chamber per dilution (Positive controls). After inoculation, the cells were put in a 37°C CO_2 cell culture incubator and incubated for one hour for viral adsorption. After adsorption, the inoculum (from highest to lower dilutions) was removed from each chamber with Pasteur pipettes, and the cells were rinsed twice with dilution and maintenance medium. Finally, 1 ml of fresh-prepared dilution and maintenance medium was added to each chamber, and incubated in the 37°C CO_2 cell culture incubator. Cytotoxicity and virus-induced cytopathic effects (CPE) were accomplished twice daily. At 48 hours (2 days) after inoculation, the culture medium was removed and the cells were fixed in each chamber with 70% acetone for 20 minutes at -20°C. The slides were air dried after fixation and stored at -20°C before staining. Before IFA staining, the upper plastic structures and gasket were removed from each slide, and rinsed briefly in PBS. Next, 1-2 drops of optimal dilution of swine anti-TGEV hyperimmune serum (diluted in 1X PBS) was added to each well and gently spread to cover all the cell surface area. The slides were incubated in a humidified chamber for 30 minutes at 37°C. The TGEV serum was rinsed off 3 times in PBS with 10 minutes of soaking time each. The PBS was blotted from the slides, and 1-2 drops of optimal dilution of Fluorescein-labeled affinity purified

antibody to swine IgG (H+L) was added to cell monolayers of each chamber and spread to cover all the cell surface area. The rinsing and blotting steps were repeated. Next, 1 drop of mounting medium was added to the cell monolayer in each chamber, and covered with coverslips. The slides were read using a UV microscope (Nikon Diaphot 200). A cell was considered IFA-positive when its cytoplasm was fully fluorescence-stained. For a valid test, cells in the TGEV inoculated chambers should be IFA-positive, and cells in the medium-control chamber should be IFA-negative. TGEV infected cells demonstrated a typical cytoplasmic fluorescence.